

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF BACTERIOLOGY
BERKELEY 4, CALIFORNIA

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Dr. Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison 6, Wisconsin

Dear Josh,

Thanks for your most interesting letter. I have given your suggestions much thought, with the following results:

1) You suggest plating irradiated cells on complete agar, incubating several hours and then replicating to penicillin agar. Do you mean that the velvet will pick up colonies as small as 10 - 100 cells? I thought that macroscopic colonies would be needed to insure transfer.

2) I think the use of the replicate plate technique throughout this procedure is undesirable, since it requires four times the number of plates. Using it in the last step only, with introduction of earlier supplements under the agar, is a nice idea, but the advantage of avoiding picking submerged colonies to a "master" plate for later replication is balanced by the technical awkwardness of the injection process - something I never could do reproducibly and uniformly.

3) I like your idea of eliminating penicillinase. We are trying combinations of cysteine and high pH this week to inactivate the penicillin.

We are excited about the "velvet" method, and have been playing with it for two weeks. We have done experimental transfers of a variety of mutant types on hand, and have been quite pleased except for one thing: a "vitamin mixture" mutant (we haven't screened it further yet) is always negative when streaked from suspension on washed mineral agar, but will grow on washed mineral through as many as 8 successive replications* with the velvet. We use a master plate which has 36-hour old transfer spots on it. Apparently carry-over is serious, here, although other mutants, including another vitamin-deficient strain, worked well. Do you have any suggestions about avoiding carry-over? I could send you the guilty strain, if you like. By the way, streaking the bug on minimal which had been pressed with the velvet cylinder after first pressing the cylinder on sterile complete agar did not yield growth. Thus the carry-over comes with the organisms.

Best regards to Esther,

Sincerely,



Edward A. Adelberg

EAA:hls

* Later experiments with 72-hr old colonies replicated positively only twice on minimal.